

## ISLET IMAGING WITH THE LEICA DFC490 CAMERA

### Abbreviations and Codes

IEQ                                      Islet Equivalents

### Safety Routines

All work with human material always carries a risk for transferring disease. See [Skyddsföreskrift laboratoriearbete KITM AL4731](#) and [Hygienregler för Akademiska sjukhuset AL9112](#).

### Procedure

*Note: It is difficult to analyze pictures if there is too much tissue or if there is too much red dithizone color.*

1. Make sure the QWin/Runner USB key (purple) is in a USB port of the computer used to take pictures.
2. Open Leica Qwin.
3. Click on the Image Preview button. Alternatively, one may open the menu option Image and then select Image Preview. An image should appear on the screen (with settings panel on left side).  
Note: Make sure the microscope viewer is set to PHOTO not BINO.
4. Place the dithizone-stained sample on the microscope and focus. It is easiest and quickest to turn the light on the microscope, reduce exposure time (e.g 10-100 ms) and then focus. Ensure the maximum amount of tissue is taken in the picture.
5. Turn the microscope light off and set exposure time to approx. 2 seconds. If Protect the samples from direct light, use the white plastic column mounted on the microscope or use a plastic embedded white paper to soften the light. Adjust exposure time or other settings if necessary.
6. Press the Acquire button. While the picture is being taken prepare other samples by swirling the tissue to the center of the dish.
7. When the picture has been taken save the picture as a jpeg file in a folder created for the specific pancreas (e.g. G:\KITM\Enh Cellulärimmunologi\Ö-lab\Bilder – humanisoleringar\Hxxxx\Iso ). Use the human number and the COBE fraction number for the file name (e.g. "H1234 C1.1 iso" for the first COBE fraction 1 or "H1234 f1 iso" for fraction 1, etc). Create new folders when necessary and place them at G:\KITM\Enh Cellulärimmunologi\Ö-lab\Bilder – humanisoleringar\Hxxxx\Iso.
8. Repeat the procedure 3-7 to take all pictures.

**Fotografering av öar med Leica DFC490 Islet photography with Lieca DFC490 710966**

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9. If needed it is possible to remove the red dithizone solution to improve image quality by using a syringe and needle of 16G or larger. Ensure the tissue is in the middle of the petri dish. Draw slowly up the supernatant into the syringe while moving the needle point along the edge of the dish. Avoid drawing up tissue. Fill the dish with Ringer's acetate and work the tissue to the middle of the dish. Repeat steps 3-7 again.
10. Estimate the sample purity as a percentage of stained tissue compared to the total amount of tissue (red colored islets and the yellow-brown/white exocrine material). Enter the purity into the database. Alternatively, it is possible to estimate sample purity using the program Qwin Runner according to the protocol *Digital Imaging Analysis of Isolated Pancreatic Tissue 710967*.

### Calculations and Protocol

The total islet equivalents (IEQ) calculated according to the following formula:

$$\text{IEQ} = \frac{\text{percent purity} \times \text{the volume of the pellet expressed in } \mu\text{l} \times 1000}{230}$$

This equation is built into the NICS database fields for calculating islet numbers.

### Equipment

Inverted Microscope (Nikon TS100) with camera (Leica DFC490)	MTA 075959
Computer with Leica QWin installed	086831

### Material

Pipet, 2 ml plastic sterile	746063
Petri dish, Sterilin 50 mm	746201
Pipet-boy	746253

### Reagents/Additives

Ringer's Acetate	767600
Dithizone working solution	767596

### Good to know

It is difficult to analyze pictures if there is too much tissue or if there is too much red dithizone color. Dithizone working solution expires after 2 weeks from reconstituting the powder if protected from light.

### References

[Bestämning av antalet ö-ekvivalanter \(Estimation of total islet equivalents\) AL5173](#)

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[Odling av öar \(Islet culture\) AL5203](#)

[Skyddsföreskrift laboratoriearbete KITM AL4731](#)

[Hygienregler för Akademiska sjukhuset AL9112](#)

[Digital bildanalys av isolerad pankreasvävnad AL5478](#)

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